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Product datasheet for AM26597AF-N

XIAP (352-449) Mouse Monoclonal Antibody [Clone ID: 2F1]

Product data:

Product Type:	Primary Antibodies
Clone Name:	2F1
Applications:	WB
Recommended Dilution:	**Western blot:** 1 µg/ml for chemiluminescence detection system. For details see protocol below. This antibody has also been used in immunohistochemistry (paraffin-embedded and frozen sections) and immunocytochemistry at 1:75 dilution (see Ferreira, et al, 2001).
Reactivity:	Human, Mouse
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	GST-XIAP fusion protein corresponding to full length amino acids (1-497 a.a)
Specificity:	This antibody reacts with XIAP by Western blotting. 2F1 recognizes the C-terminal region of XIAP (a.a. 352-449) and detects the 54 kDa XIAP on Western Blots using total cell lysate (for example Jurkat, Raji, NIH/3T3).
Formulation:	PBS containing 50% glycerol. Contains no preservatives. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein-A Sepharose
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	X-linked inhibitor of apoptosis
Database Link:	Entrez Gene 331 Human P98170

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Background: Caspase, related to ICE and to CED-3, play a central roles as effectors of apoptosis. Ablation of caspase activity is attained by P35 from baculovirus and CrmA from cowpox, which appear to be suicide inactivators, strongly inhibiting caspase activity. Overexpression of there caspase inhibitors in insect, nematoda and mammalian cells results in resistance to apoptotic stimuli, demonstrating that components of the apoptotic pathway are highly conserved throughout evolution, and leading to the speculation that mammalian functional equivalents of these protease inhibitors may exist. The inhibitor of apoptosis (IAP) are a family of antiapoptotic proteins that are conserved across species. Four IAPs have been identified in mammal; NAIP, X-IAP/hILP, c-IAP-1/HIAP-2/hMIHB, and c-IAP-2/HIAP-1/ hMIHC. A prototype of the human IAPs is the X-IAP, with a 1.5-kb coding region corresponding to a 55-kDa protein. XIAP can directly inhibit two members of the cell death protease family, caspase 3 and 7.

Synonyms: HILP, API3, XIAP

Note: This product was originally produced by MBL International.

Protocol:
SDS-PAGE and Western Blotting
1) Boil all samples for 3~5 minutes. Load 10 µ l of cell lysate or tissue homogenate (5~20 µ g total protein) to each well of an SDS-polyacrylamide gel and electrophorese in a 1 mm thick gel.
2) Transfer to a polyvinylidene difluoride (PVDF) membrane at 10V for 1hour in a semi-dry transfer system. (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH).
3) The transferred proteins can be visualized by staining the membrane for 1 minute with Ponceau S. Rinse the membrane with PBS.
4) Non-specific binding sites are blocked by immersing the membrane in 5% Skim Milk / PBS / 0.05% Tween20 for 1 hour at room temperature, or for overnight at 4°C.
5) Incubate in primary antibody diluted as suggested in the APPLICATIONS for 1 hour at room temperature. (The optimal concentration of the antibody will depend on the conditions used.)
6) Wash the membrane 3 times with PBS, 0.05% Tween20 for 5~10 minutes per wash.
7) Incubate in horserad ish peroxidase conjugated with goat anti-mouse IgG diluted 1:3000 in PBS, 0.05% Tween20 for 45 minutes at room temperature.
8) Wash the membrane 3 times with PBS, 0.05% Tween20 for 10 minutes per wash .
9) Incubate in Amersham ECL Reagent for 1 minute. Drain membrane, remove excess ECL Reagent by dabbing with a Kimwipe, and seal in plastic wrap.
10) Expose to ECL hyperfilm in a dark room for 30 seconds. Develop as usual for autoradiogram or X-ray. The conditions for development and exposure may vary.